Appl. No.: 10/507,355 inventor: Klaus K. NIELSEN et al. Docket No.: 0147-0262PUS1

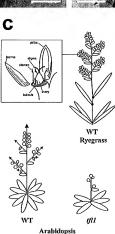
Title: METHOD OF REPRESSING FLOWERING IN A PLANT Sheet 1 of 14

REPLACEMENT SHEET

1/14 FIGURE 1







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Inventor: Klaus K. NIELSEN et al.

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	GCC	-76
-75	${\tt CAAGCCACTTCAAAGCTTTGCTACTACCAGATAGAGCATTCACCGTGCAATATAGAAATACTTGCCTCTCCAACCCTCCCAACAAACAAACAAACAAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAAAA$	-1
1	ATGTCTAGGTCTGTGGAGCCTCTTATTGTTGGTCGTGTCATTGGAGAAGTTCTCGATCCATTTAACCCATGTGTG	75
76	AAGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCATGAGCTCTACCCATCAGCAGTTGTATCT	150
151	AAACCAAGAGTAGAGGTTCAGGGGGGTGACTTGCGATCCTTATTCACATTGGTTATGACGGACCCAGATGTGCCA	225
226	GGACCAAGTGATCCGTATCTGCGGGAGCATCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCA	300
301	$\tt TTTGGGGGGGGGGGTCATGAGCTATGAGGCCCAAAGCCCAACATTGGAATCCACAGGTTCATTTTTGTGCTCTTC$	375
375	AAGCAGAAGCGAAGGCAGACTGTATCTGTGCCTTCCTTCAGGGATCATTTCAACACCCGGCAGTTTGCTGTGGAT	450
451	${\tt AATGATCTTGGCCTCCTGTGGCTGCTGTTTACTTCAATTGTCAGAGAGAG$	525
526	${\tt TCGAGTTCTTGGCTATCCCAGTTGTGCCAAATAAAGGCTTTTGGAGTTATGCACCTTCTTTCT$	600
601	${\tt CCTCTTCTACATTACTTCCTCGTGGACCATTGCTTCTTTACTACAGTTTTTGCTCAGGGATCAAATAAAT$	675
675	${\tt GCATTTTGGAGATTGTATTATATTGTAAGCAGTGAGATCAGCAACCATGTGTTAACATAAGCCAGTACATAGCCAGTACATAGCCAGTAGAGTGTGTAACATAGCCAGTAGATAGCCAGTAGAGTGTGTAAGAGTGAGAGTGTGTAAGAGTGAGAGAGTGAGAGTGAGAGTGAGAGAGTGAGAGAGTGAGAGAGTGAGAGAGTGA$	750
751	TAGCAGGTCCATGTTTATGGTTTCATGTTGTGTGTAAGCAGTTATCACTAGAAGGAAG	825
826	A BOTT COURS A BABBA BAB COTTT BTOTA	851

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FIGURE 3A -3600 cactagtaacqqccqccaqtqtqctqqaattcaqqqtaatacqactcactataqqqmqctcqaqqatcttcccac -3526

-3525 cagtgtgcattcatgtgttacttaccactctccaacttgagggactcaagattggtggggggctccttttcgctg -3451 -3450 aagcgatccaaaggtqtcqqqtaacqqttatqacaqcaaacaqaaacatcqccatctqcacqqaaqccaqaagt -3376 -3375 agttactatgtcaaagggatataaaaaactcactaatgaagggggatgtattgctgagataaactgctatctca -3301 -3300 totacagqtqaqattqcaaqtatacttqacaacaqqqccaqatqqtatqqcatqaaqaaaattagqqctqqaqta -3226 qaaaqqtaaqatatgcatggatttggatgagatggctagaggttgcgagatatcaaatagaaqacacttcttca -3151 -3225 -3150 atgattcaatagaagatgcatqtqcattacaqagtgqattattatqtcctttttaaagagatqcttacqtccct -3076 gacctttcctataacacaattacactcctttgctagacttttcctgctataattgtctttcctcgccaaagaat -3001 -3075 -3000 aatactatagaacttcctaatttaatttccccttattttcttggactctatcttaattctcctcctattgttcag -2926 -2850 octgaatetattteteaceteatgetgeaatgeteetteleacageaaatatggtatgatatetgeagtaagete -2776 -2775 aacettetgecatgtatgecagttggcaacgecgagtteageatttggttegecgcagetgecgecaacgeteaa -2701 -2700 ccagcctgcagaagggtgctaaatccatcatcatccttactcttggagattatggaagacgaggaacgatgct -2626 -2625 atottoaasaatotggccccaacagactcqccttagttcagtcgatoctagatgaagcctgtcaatggtcgtta -2551 -2550 gccggtgctaaggcgctacgtcagttacctttacatgctagacccctgatgttagccttgatgaggaactctag -2476 -2325 qcatqtqttcqaqaaaaaatttacttacctcttaggctatattctcttcaccaacttggactccacaaagcttc -2251 -2250 aategeaacttgtccaagetgctgctgctgctgctgtcttttccaatqcatccatacactgtcctaqtcaq -2176 -2175 cataccaaccaacaaaaaqctaatqccqccctqttqtttcaaatqaattatctqattqtqatqctqctaatctttt -2101 -2100 gcatatgagtctcgggcatatgaatgaacttggtttggcagaatgaaacaagagaggacttcttgatggatatag -2026 -2025 cactqqtaaqctqaaqttctqtqaqcaqqctatqatgttcccctgttaaaaaaaaggctatgaaaaacttgtgat -1951 -1950 aggraphiaagtattggttttattttgggtggaaattggtatggaaagttgtagtgctactagtctgtggtg -1876 -1800 atteatogacccattttgttataattttcttttaaastaaasattccgtaaagaatcaataagtggaattattg -1726 -1725 qaaatqaaaaaqtaaccaaaatactaaactttttttcaaatacqatcqqatatcatqqaqacacactqqctac -1651 -1650 cattggttggaatagctactagattccactacagctaggtgtcaagcaactataatggcatcagaatggagcaga -1576 -1575 aaaatgtcacaagctgtacttcactccactacttctagctgcacaaatgtcaagcaggcatgattgcactagacc -1501 -1425 aatcqaqatqaaqctqtqataattttatcqctqaaatqacatttcaqcactaqacaqcaccctaqacaattaaqt -1351 -1350 qqtqqtqqcactqtattccattcctttattctcttccatqqtqtttcccataqtactacaaaqaaqaataaa -1276 -1275 cagataataatagtaatgcacttqqqtatcqaaqttttaqqaaaqattctaattctaqaqcaattqaactcaaca -1201 -1200 acaacttcccttttccttaacagaaaagaatcggtcaaacgaggcttgcctaaaccaacaacactataaagacg -1126 -1125 aacatttqaqqqtqaaqaqqcttccacqtqqacaqtqccqcatqtttctqtccactaqataacacctaaataata -1051 -975 caaaaacqaattqataqtttaqqaaqqaatcactccaaaqtqttttattcccqttctttttcatttqctccacaa -901 -900 qqqcatacttcctaaatttctgcgaacaattacatctagatctttttaaaactgaaqtattttaqcatqaaaacq -826 -751 -750 acacaacagcatgataatgtgcaaaactaagcatcaaaatctgcacattgtcatgcagaaactaggacaggagga -676 -675 ccagcactttqtcqtttqtcctaaccaatattaacataqttcaqcaacataatcttcaqaqacccactaqcatqa -601 -600 agqtqtqttatqtttcctaaaqaaataacatqtagqtagtqatctacaataccttttttggggactataaggtgg -526 -375 catotttctggtgaaaaaattctctgcccctagaacttggaagaaqatgcatgaagtattactccaaactccaac -301-300 actqtqcaactqataqaaaaqaaacaaqaccttgqttqqctqtctcqqaaaaaqtgqttagqtcctttctqtgg -226 -151 ~150 gcttacactcqaagcaggcttcttgcctctataagtagaggctcgtcgtcctctagcaatgctcagtaagcaGCC -76 -75 CAAGCCACTTCAAAGCTTTGCTACTACCAGATAGAGCATTCACCGTGCAATATAGAAATACTTGCCTCTCCAACC ~1 1 ATGTCTAGGTCTGTGGAGCCTCTTATTGTTGGTCGTGTCATTGGAGAAGTTCTCGATCCATTTAACCCATGTGTG 76 ABGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCATGAGCTCTACCCATCAGCAGTTGTATCT 150 151 AAACCAAGAGTAGAGGTTCAGGGGGGTGACTTGCGATCCTTATTCACATTGgtagaatgcactcgactcgatctt 225 quaactccatattcaacttcqaqtattqtatqcttqttittcttcttcqcaqtqqccataattattcatatttca 300 301 gottatgacggacccagatgygccaggaccaagtgatccgtatctgcgggagcatcttcactggtaacetttete 375 450

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Inventor: Klaus K. NIELSEN et al.

INVENIOR: NIGHTS AND A PLANT
TITLE: METHOD OF REPRESSING FLOWERING IN A PLANT
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FIGURE 3B

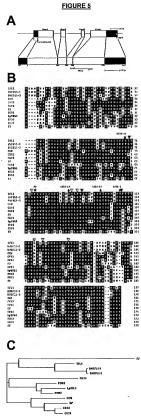
451	tagtatatgttgctaaggaatatagaagtacatcttcttcttgcacatatatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactatttttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagactattttaatagacagagagacagagagactattttaatagacagagagacagagagacagagagacagagagacagagagagacagagagagacag	525
526	$cacttctaac gagagtcatttaccaatacctttacacttacacagg \verb ATTGTCAGTAATATACCTGGGACAACAG \\$	600
601	${\tt ATGCTTCATTTGG} taggtcottctctgagatttgaattggtatattctatgttctgcattttgaatgaa$	675
675	$\verb ctgaccttttgaattgcaggggggggggggggggggggg$	750
751	${\tt ATTTTTGTGCTCTTCAAGCAGAGCGAAGGCAGACTGTATCTGTGCCTTCCTT$	825
826	${\tt CAGTITGCTGTGGATAATGATCTTGGCCTCCCTGTGGCTGCTGTTTACTTCAATTGTCAGAGAGAG$	900
901	${\tt AGGAGGCGCTGARAATCGAGTTCTTGGCTATCCCAGTTGTGCCAAATAAAGGCTTTTGGAGTTATGCACCTTCTT}$	975
976	${\tt TCTGAAGTCAATGCTCCTCTACATTACTTCCTCGTGGACCATTGCTTCTTTACTACAGTTTTTGCTCAGGGA}$	1050
1051	${\tt TCARATAAATCAAGTGCATTTTGGAGATTGTATTAGATTATATTGTAAGCAGTGAGATCAGCAACCATGTGTTAA$	1125
1126	${\tt CATARGCCAGTACATTAGCAGGTCCATGTTTATGGTTTCATGTTGTGTGTAAGCAGTTATCACTAGAAGGAAG$	1200
1201	${\tt CAGGTAGACCCAAACTGGCAAAAAAAAAAAGCTTTATCTActgtatggcccttgccggcttgatgttccatgc}$	1275
1276	accttttctgacatgctgtctactgtatgccaccgccactataatgtatgagatatgaatataaaatggagatat	1350
1351	${\tt ccassatatccagatgattgcccactasatgctasatgtacatagtgggttttccacctattttgacttcatcat}$	1425
1426	$\tt gtccttacacaaaatcagaaaacatccatttcatgcacattgatgcacactgcatattaacaatctattcagatt$	1500
1501	tggctgtaaacacacccttattttccgcatccattaatattatattagtaccctggacaggttaagcttttgcag	1575
1576	cacagtaagtaaccggatgaaattacaatatgatcctcgagcgccctat	1624

1	MSRSVEPLIVGRVIGEVLDPFNPCVKMVATYNSNKLVFNGHELYPSAVVSKPRVEVQGGDLRSLFTLVMTDFDVP	75
76	GPSDPYLREHLHWIVSNIPGTTDASFGGEVMSYESPKPNIGIHRFIFVLFKQKRRQTVSVPSFRDHFNTRQFAVD	150
151	NDLGLPVAAVYFNCORETAARRR	173

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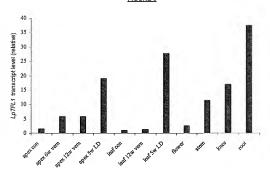


Appl. No.: 10/507,355 Inventor: Klaus K. NIELSEN et al.

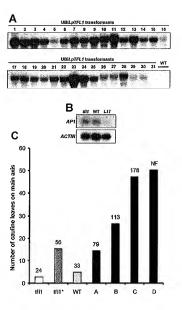
Docket No.: 0147-0262PUS1

Inventor: Klaus K, NIELSEN et al.
Title: METHOD OF REPRESSING FLOWERING IN A PLANT
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6/14 FIGURE 6



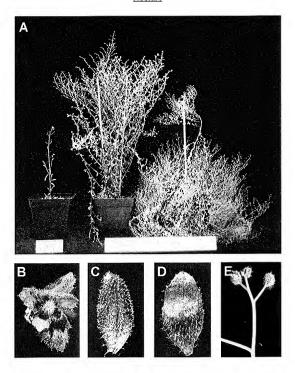
Appl. No.: 10/507,355 Docket No.: 0147-0262PUS1 Inventor: Klaus K. NIELSEN et al.
Title: METHOD OF REPRESSING FLOWERING IN A PLANT REPLACEMENT SHEET Sheet 7 of 14 7/14



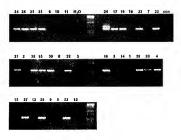
Docket No.: 0147-0262PUS1

Appl. No.: 10/507,355 nventor: Klaus K. NIELSEN et al. Title: METHOD OF REPRESSING FLOWERING IN A PLANT Sheet 8 of 14 REPLACEMENT SHEET

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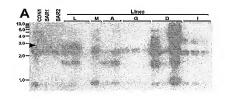
Appl. No.: 10/507,355 Docket No.: 0147-0262PUS1 Inventor: Klaus K, NIELSEN et al. Filte: METHOD OF REPRESSING FLOWERING IN A PLANT PEPLACEMENT SHEET Sheet 9 of 14

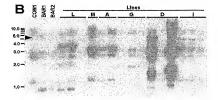


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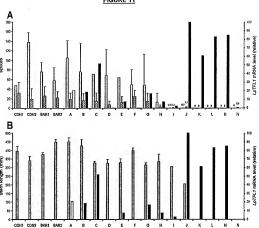


FIGURE 12

							UBI::LpTi	Lilling	3				
	CONI	c	Α.	J	L	м	D	1	N	н	В	E	к
LpTFL1 mRNA		ఱ	220	瓣	-	-						1	
LPACTINIMRNA	柳田	100	480		1	-	400			**	200		
MS56 LP4REV	69090 8. 252					e busine		al grains	N.		Printer.	Maria I	

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FIGURE 13

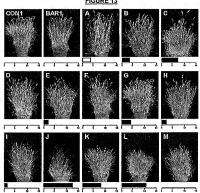
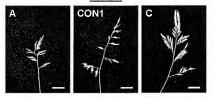


FIGURE 14



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FIGURE 15:	Transformation	Efficiency and Floral	Activity of the	Transforma
Cultivar	Line No.	Inflorescences	PCR	RT-PCR
F6	CON	8	-	-
F6	7	18	-	-
F6	8	11	-	-
F6	17	5,3	+	-
F6	18	13,3	+	-
F6	24	12	+	+
F6	29	0	+	+
F6	32	0	+	+
F6	33	4	+	+
F6	36	0	+	+
ACTION	2	1,8	-	-
ACTION	5	3	-	-
ACTION	9	0,3	-	-
ACTION	12	2	-	-
ACTION	13	0	-	-
ACTION	16	0	+	-
ACTION	19	7,3	+	-
ACTION	21	4	+	+
ACTION	22	0,3	+	+
ACTION	23	0	+	+
ACTION	25	0,3	+	+
ACTION	27	0	+	+
ACTION	28	4	+	+
ACTION	31	0	+	+
ACTION	34	0	+	+
ACTION	35	0	+	+
TELSTAR	1	10	-	-
TELSTAR	3	1	-	-
TELSTAR	4	11,6	-	-
TELSTAR	6	10,8	-	-
TELSTAR	10	5	-	-
TELSTAR	11	3,8	-	-
TELSTAR	14	0		-
TELSTAR	15	3,8	+	_
TELSTAR	20	3,5	+	-
TELSTAR	26	0	+	+
TELSTAR	30	3,7	+	+

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Primer combination				_	JBI::Lp	TFLI	UBI::LpTFL1 transgenic linesa	ic line	es.						
CASSETTE ULizonaria Intron Estate	CON BAR A		ВС	Q	ш	ш	O	Ħ	-	-	×	٦	Σ	z	A
				0.8						+		+		8.0	2.3
M833-LP4REV				0.55						+		+		0.55	2.0
M831-LP4REV		+		+			4.1	+		+		+	+		1.5
MS56-LP575			+	+/0.5	+		+/0.5	+	+	+	+	+	+	+/0.5	9.0
SSW-DGT		T	+	W/+	+		+	+	₹1.8	+	+	+	+	+/1.6	9.0
MSS6-LP4REV (PROSELPTEL F probe)		+	+	+	+	+	+	+	+	+	+	+	+	+	4.0
Promoter	8	short		TATA			short	short short		쏭		쏭	short	ok short TATA box	
Result		성		쓩			쏭	쓩		성		상	ø		

extra Plus indicates that the observed fragment had the expected size, whereas numbers indicate that the fragment size deviated from the expected size (numbers in bold), blank field indicates that no PCR-product was detected; E, EcoRi; H, HindIII ok + extra trun-cated ok + extra trun-cated

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쑹 쑹

쏭

ķ 쏭

LpTFL1 cDNA

Figure 16: Transgene integration analysis by PCR using different primer combinations